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Synthetic Catalysts that Hydrolyze Phosphate and Carboxylate Esters

Anthony W. Czarnik
Department of Chemistry

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Office of Naval Research
Arlington, Virginia 22217-5000

Contract No. N00014-91-J-1869
Final Report

September 1992

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a REPORT SECURITY CLASSIFICATION Unclassified			1b RESTRICTIVE MARKINGS		
2a SECURITY CLASSIFICATION AUTHORITY			3 DISTRIBUTION/AVAILABILITY OF REPORT approved for public release; distribution unlimited		
2b DECLASSIFICATION/DOWNGRADING SCHEDULE					
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Report #11			5 MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Ohio State University		6b. OFFICE SYMBOL (If applicable) OSU	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research		
6c. ADDRESS (City, State, and ZIP Code) Department of Chemistry 120 W. 18th Avenue Columbus, OH 43210-1173			7b. ADDRESS (City, State, and ZIP Code) Arlington, VA 22217		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research		8b. OFFICE SYMBOL (If applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-91-J-1869		
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000			10 SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO 61153N	PROJECT NO RR1309	TASK NO 413p
					WORK UNIT ACCESSION NO 413P003
11. TITLE (Include Security Classification) Final Report for Contract N00014-91-J-1869					
12. PERSONAL AUTHOR(S) Anthony W. Czarnik					
13a. TYPE OF REPORT final		13b. TIME COVERED FROM 3/91 TO 2/92		14. DATE OF REPORT (Year, Month, Day) 1992 Sept 18	
15. PAGE COUNT					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
07	03				
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
This is the final report for contract N00014-91-J-1869.					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Harold E. Guard			22b. TELEPHONE (Include Area Code) (202) 696-4409		22c. OFFICE SYMBOL ONR



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Contract No. N00014-91-J-1869
Final Report
RF Project No. 769222/725072

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Approved For	
DATE 10/21/92	<input checked="" type="checkbox"/>
BY 11/18	<input type="checkbox"/>
Classified	<input type="checkbox"/>
Declassification	

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OFFICE OF NAVAL RESEARCH
FINAL REPORT

R&T Number: 413p003---04

Contract/Grant Number: N00014-91-J-1869

Contract/Grant Title: Synthetic Catalysts That Hydrolyze Phosphate and
Carboxylate Esters

Principal Investigator: Anthony W. Czarnik

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(1) Statement of objectives

We are looking for reaction types that simultaneously: (1) provide for the reaction of acyl and phosphoryl groups under non-forcing conditions; (2) suggest ways for elaboration into catalytic cycles with turnover behavior; and, (3) survive translation onto binding moieties. To date, we have focussed on artificial metalloenzymes derived from Co(III) and Cu(II) coordination complexes with cyclodextrins, preassociating α -nucleophiles, and binuclear metal ion complexes

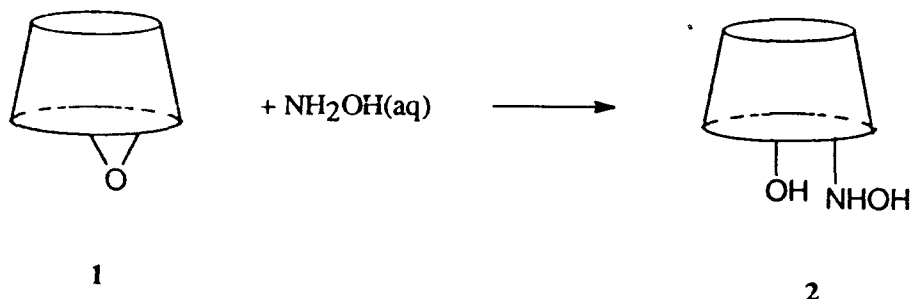
*(2) Statement of accomplishments***PREASSOCIATING α -NUCLEOPHILES**

Cyclodextrins have been prepared bearing imidazole as a group with reactivity at pH 7; pendant coordination complexes have likewise been employed. However, as potential pendant groups, α -nucleophiles such as hydrazine or hydroxylamine offer unique properties. (1) In solution, α -nucleophiles show enhanced reactivity towards acyl transfer as compared to isosteric alcohols or amines. (2) Despite their greater reactivity towards acyl compounds, hydroxylamine (pK_a 5.97) and hydrazine (pK_a 8.0) are less basic than isosteric amines (pK_a 9-10), and thus exist in a reactive form near neutral pH. (3) Both hydroxylamine and hydrazine transacylate alkyl esters and amides. (4) Because they are physically small, pendant α -nucleophiles would necessarily reside proximal to the CD binding cavity.

In our last report, we described the syntheses, characterizations, and reactivities of primary-side derivatives β CDNHNH₂ and β CDNHOH. Both β CDNHNH₂ and β CDNHOH are acylated rapidly by *p*-nitrophenylacetate (pNPA) with saturation behavior. The reaction of pNPA (0.05 mM) fully complexed to 5 at pH 7.0 and 25°C is faster than that with equimolar CH₃NHOH ($k_2=1.0 \text{ M}^{-1} \text{ s}^{-1}$), demonstrating an effective RNHOH concentration of 37 mM. β CDNHOH is acylated as efficiently at pH 7.0 as at pH 9.5; furthermore, the rate of acyl transfer is 1500-times faster than that afforded using equimolar β CD, which is not reactive under neutral conditions. This work has now appeared in print (pub. 7).

More recently, Mark Mortellaro of this group has synthesized and studied the corresponding secondary-side hydroxylamine derivative. 2°- β CDOTs was prepared from β -cyclodextrin (β CD) according to the procedure of d'Souza and was converted to the β CD-manno-2,3-epoxide (1) by stirring in aqueous ammonium bicarbonate solution. Passage of 1 through a Dowex

mixed bed ion exchange column removed unwanted salts. A solution of 1 in a 50% aqueous solution of hydroxylamine was stirred overnight under argon at ambient temperature. Two precipitations from ethanol removed free hydroxylamine and gave 2 as a colorless solid in 62% yield. TLC showed



spots at R_f 0.28 for the monohydroxylamino cyclodextrin and at R_f 0.22 for one or more (presumed) dihydroxylamino cyclodextrins. Elemental analysis of the product sample likewise established a ratio of 1.4 nitrogens per CD unit, corresponding to a mixture of 60% mono- and 40% di-hydroxylamino cyclodextrin. Diaxial ring opening of the epoxide by hydroxylamine is predicted by the known reactivity of 1 and of related glucose derivatives.

Rate constants for the reaction of *p*-nitrophenylacetate (PNPA) with 2° - β CDNHOH, 1° - β CDNHOH, CH_3NHOH , and β CD were measured at several pH values (Figure 1). As is apparent from Figure 1, 2° - β CDNHOH is less reactive towards PNPA than is 1° - β CDNHOH under identical conditions.

However, the reactivity of 2° - β CDNHOH is pH dependent (a 35-fold increase from pH 6.5-9.5) while that of 1° - β CDNHOH is virtually pH independent over the same range (a 1.6-fold increase). Assuming that substrate binding to the cyclodextrins is largely unaffected by pH in this region (likely given the pK_a 's involved), then the contrasting reactivities must be explained in terms of how the hydroxylamine units can interact with the cyclodextrin framework. The pH-independent rate profile of 1° - β CDNHOH parallels that of CH_3NHOH , reasonable if the 1° -side of the cyclodextrinyl ring does not influence the acid-base microenvironment of the attached NHOH group. The rate acceleration of the 1° - β CDNHOH over CH_3NHOH is thus due entirely to preassociation of the substrate.

Alternatively, the pH-rate profile of the 2° - β CDNHOH is indicative of catalysis by base. We interpret this pH effect on 2 as evidence that the 2° -side of the cyclodextrinyl ring influences the reactivity of the attached NHOH group. While the pK_a of a 2° -OH is about 15-16, the first pK_a of β CD's vicinal diol is 12.1 as a result of intramolecular hydrogen bonding. β CD itself is well-known to be inert at neutral pH (the β CD rate in Figure 1 is attributable entirely to buffer catalysis); rather, it demonstrates base-catalysis only above pH 10, when its 2° -hydroxyl groups have begun to deprotonate. CPK models suggest hydrogen bonding is similarly possible between the pseudoequatorial C-2 hydroxyl and the C-3 hydroxylamine groups of 2. This work has been accepted for publication (pub. 8).

Reactions of PNPA with Selected Nucleophiles at 25°C

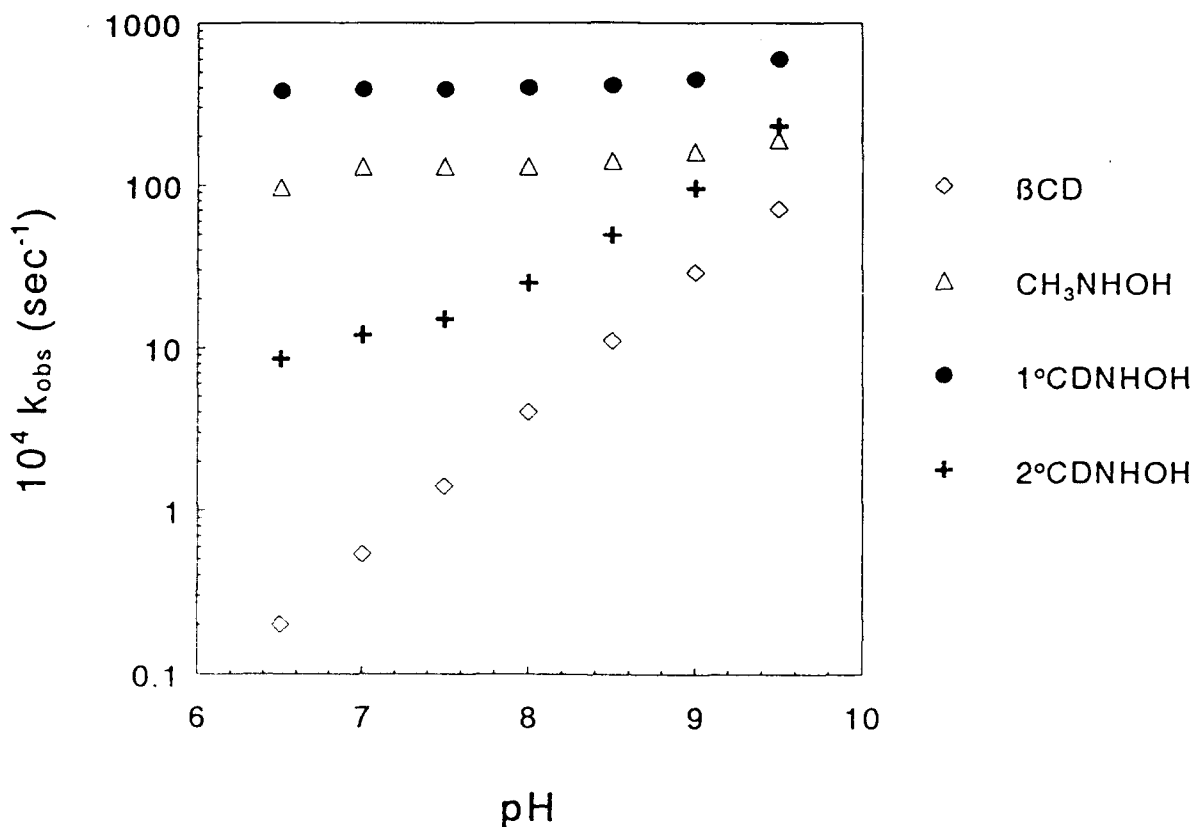
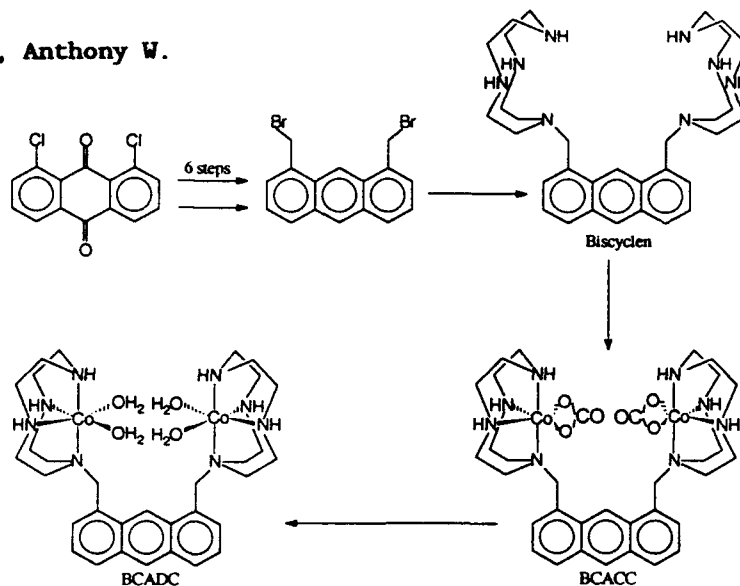


Figure 1. Observed rate constants for the reactions of the nucleophiles shown (10 mM) PNPA (50 μM) in 0.10 M bis-tris-propane buffer at 25°C. In each case, the formation of p-nitrophenol was followed at 398 nm.

METALLOENZYME MIMICS

We have prepared isomeric cyclodextrin/Co(III)-azamacrocyclic conjugates. The primary derivative increases the rate of p-nitrophenylacetate hydrolysis by a factor of 900-fold. Equally significant is the fact that the acceleration is observed at pH 7, at which cyclodextrin itself shows no activity. The full paper account of this work has now appeared (pub. 9).

We have also prepared the binuclear Co(III)-complex depicted below, which effects stoichiometric dephosphorylation of PNP-phosphate 10-times faster than two equivalents of monomeric cyclen-Co(III) complex. We are in the process of submitting this manuscript for review at the present time.

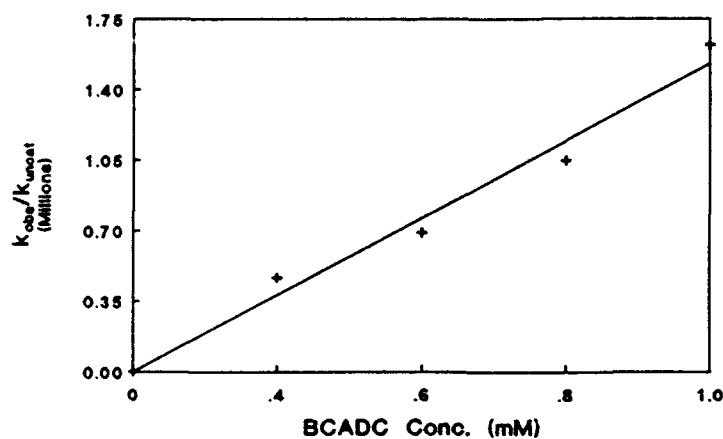


RESULTS

Phosphate ester hydrolysis with diaquo cobalt(III) complexes.
 Reactions conditions; 25°C, 0.1 M Collidine, pH 7, 25×10^{-3} mM phosphate, 2.0 mM Co(III) and monitored at 440 nm. All rates are s^{-1} .

Phosphate	BCADC	CDC	No Co(III)	BCADC/ CDC
PNPP	1.33×10^{-2}	1.27×10^{-3}	8.2×10^{-9}	10
BPNNP	1.17×10^{-4}	1.66×10^{-4}	1.3×10^{-11}	0.7

PNPP Hydrolysis by BCADC
 pH 7, 0.1M Collidine, 25°C



(3) List of publications to date resulting from this work

- 1) E. Akkaya and A. W. Czarnik, "Synthesis and Reactivity of Cobalt(III) Complexes Bearing 1⁰- and 2⁰-Side Cyclodextrin Binding Sites. A Tale of Two CD's", *J. Am. Chem. Soc.* 1988, 110, 8553.
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- 7) Lewis E. Fikes, David T. Winn, Robert W. Sweger, Morgan P. Johnson, and Anthony W. Czarnik, "Preassociating α -Nucleophiles", *J. Am. Chem. Soc.* 1992, 114, 1493.
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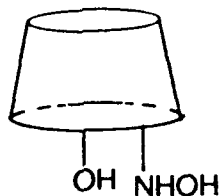
Appendices

HYDROGEN BONDING EFFECTS ON THE REACTIVITY OF A PREASSOCIATING
 α -NUCLEOPHILE. THE SECONDARY-SIDE β CD HYDROXYLAMINE

accepted

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Department of Chemistry, The Ohio State University,
Columbus, Ohio 43210

The secondary-side hydroxylamine
derivative of β -cyclodextrin
demonstrates base-catalyzed
transesterification from pH 6.5-9.5,
while the primary-side derivative does
not.



HYDROGEN BONDING EFFECTS ON THE REACTIVITY OF A PREASSOCIATING α -NUCLEOPHILE.
THE SECONDARY-SIDE β CD HYDROXYLAMINE

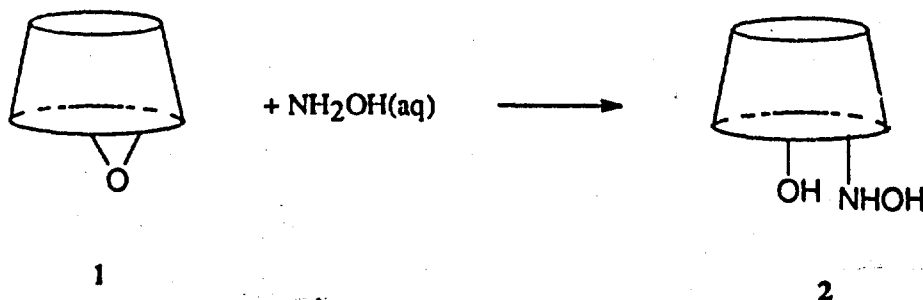
Mark A. Mortellaro and Anthony W. Czarnik*

Department of Chemistry
The Ohio State University
Columbus, Ohio 43210

Summary: The secondary-side hydroxylamine derivative of β -cyclodextrin demonstrates base-catalyzed transesterification from pH 6.5-9.5, while the primary-side derivative does not.

We have reported previously that the primary-side β -cyclodextrinyl hydroxylamine (1° - β CDNHOH) binds and transacylates both activated and less activated phenyl esters.¹ However, the primary locus of phenyl ester binding is at the 2° -side.² Furthermore, hydrogen-bonding at the β CD 2° -side is anticipated to modulate the reactivity of an appended α -nucleophile just as it does the 2° -OH of β CD itself.³ We now report the synthesis and characterization of the 2° -side β -cyclodextrinyl hydroxylamine (2° - β CDNHOH), whose reactivity is influenced by intramolecular hydrogen bonding.

2° - β CDOTs (a mixture of mono- and ditosylates) was prepared from β -cyclodextrin (β CD) according to the procedure of d'Souza⁴ and was converted to the β CD-manno-2,3-epoxide (1) by stirring in aqueous ammonium bicarbonate solution.⁵ Passage of 1 through a Dowex mixed bed ion exchange column removed unwanted salts.⁶ A solution of 1 in a 50% aqueous solution of hydroxylamine⁷ was stirred overnight under argon at ambient temperature. Two precipitations from ethanol removed free hydroxylamine and gave 2 as a colorless solid in 62% yield. TLC⁸ showed spots at R_f 0.28 for the



monohydroxylamino cyclodextrin and at R_f 0.22 for one or more (presumed) dihydroxylamino cyclodextrins. Elemental analysis of the product sample likewise established a ratio of 1.4 nitrogens per CD unit, corresponding to a mixture of 60% mono- and 40% di-hydroxylamino cyclodextrin.⁹ Diaxial ring opening of the epoxide by hydroxylamine is predicted by the known reactivity of 1^5 and of related glucose derivatives.¹⁰

Rate constants for the reaction of *p*-nitrophenylacetate (PNPA) with 2° - β CDNHOH, 1° - β CDNHOH, CH_3NHOH , and β CD were measured at several pH values (Figure 1). As is apparent from Figure 1, 2° - β CDNHOH is less reactive towards PNPA than is 1° - β CDNHOH under identical conditions.

However, the reactivity of 2° - β CDNHOH is pH dependent (a 35-fold increase from pH 6.5-9.5) while that of 1° - β CDNHOH is virtually pH independent over the same range

Reactions of PNPA with Selected Nucleophiles at 25°C

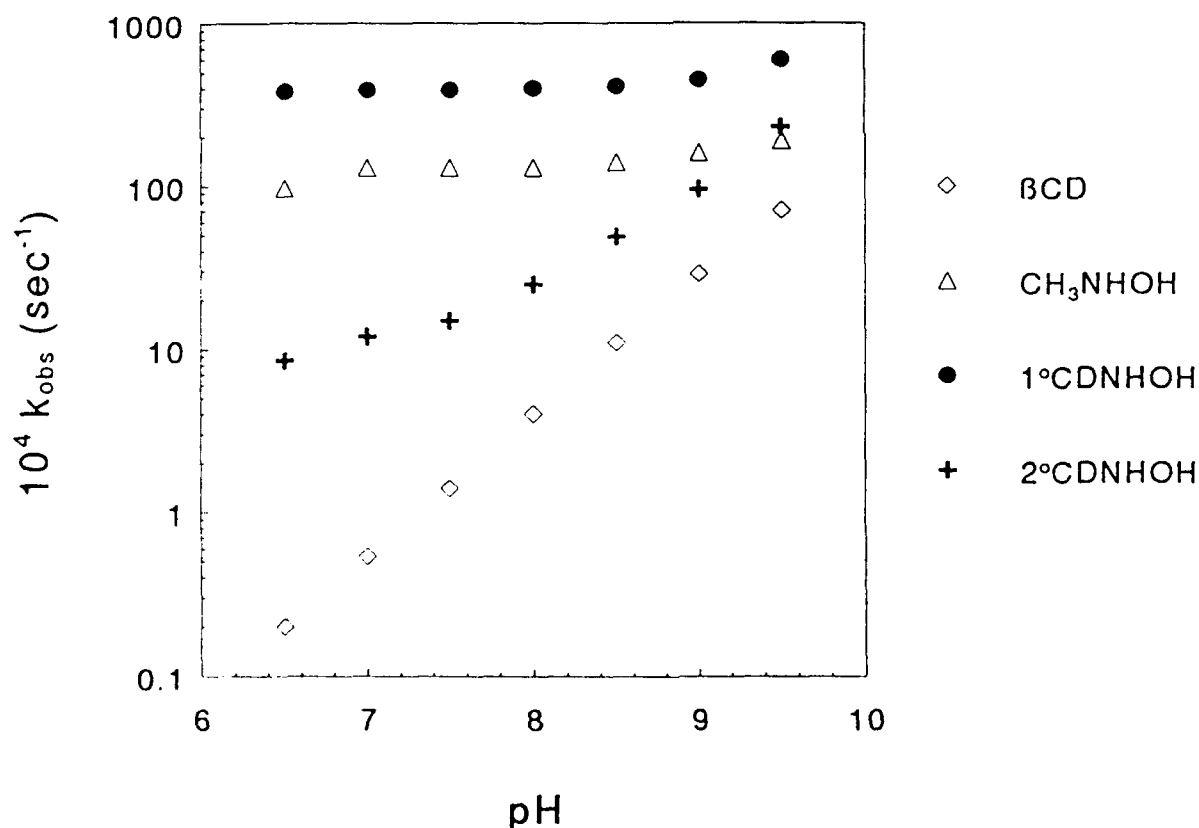


Figure 1. Observed rate constants for the reactions of the nucleophiles shown (10 mM) with PNPA (50 μM) in 0.10 M bis-tris-propane buffer at 25°C. In each case, the formation of *p*-nitrophenol was followed at 398 nm.

(a 1.6-fold increase). Assuming that substrate binding to the cyclodextrins is largely unaffected by pH in this region (likely given the pK_a 's involved), then the contrasting reactivities must be explained in terms of how the hydroxylamine units can interact with the cyclodextrin framework. The pH-independent rate profile of 1°- β CDNHOH parallels that of CH_3NHOH , reasonable if the 1°-side of the cyclodextrinyl ring does not influence the acid-base microenvironment of the attached NHOH group. The rate acceleration of the 1°- β CDNHOH over CH_3NHOH is thus due entirely to preassociation of the substrate.

Alternatively, the pH-rate profile of the 2°- β CDNHOH is indicative of catalysis by base. We interpret this pH effect on 2 as evidence that the 2°-side of the cyclodextrinyl ring influences the reactivity of the attached NHOH group. While the pK_a of a 2°-OH is about 15-16, the first pK_a of β CD's vicinal diol is 12.1 as a result of intramolecular hydrogen bonding.³ β CD itself is well-known to be inert at neutral pH (the β CD rate in Figure 1 is attributable entirely to buffer catalysis); rather, it demonstrates base-catalysis only above pH 10, when its 2°-hydroxyl groups have begun to deprotonate. CPK models suggest hydrogen bonding is similarly possible between the pseudoequatorial C-2 hydroxyl and the C-3 hydroxylamine groups of 2. Several intramolecular hydrogen bonding models involving 5 or 6 membered rings can be invoked that could rationalize the apparent base catalysis. In one such model (Figure 2), the C-2 hydroxy group hydrogen bonds to the nitrogen of the C-3 hydroxylamino group, effectively increasing the acidity of the attached hydroxyl group. The enhanced acidity of trimethylamine oxide (pK_a 4.65)¹¹ relative to that of N,N-dimethylhydroxylamine (pK_a ca. 12-13)¹² is due to the addition of a full positive charge on nitrogen. Jencks has suggested that the high reactivity of hydroxylamine towards activated esters is due to intramolecular proton transfer from the hydroxylamine oxygen to the nitrogen during attack on a carbonyl.¹³ In our model, intramolecular proton transfer to nitrogen at

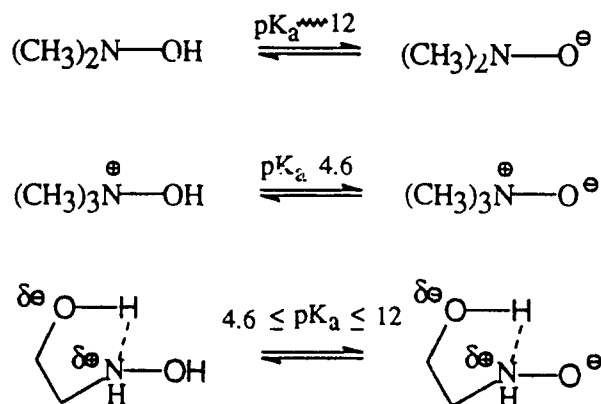


Figure 2.

neutral pH is not likely and thus consistent with the observed slower reactivity of the 2⁰-βCDNHOH compared to CH₃NHOH. To the best of our knowledge, this is the first time secondary-side hydrogen bonding has been used to modulate the pK_a of a cyclodextrin derivative. Of course, such pK_a effects on nucleophilic groups are observed commonly in enzymes themselves.

Acknowledgment. This work was supported by a grant from The Office of Naval Research. FT-NMR spectra were obtained with equipment funded in part by NIH grant 1 S10 RR01458-01A1. A.W.C. thanks the A.P. Sloan and Dreyfus Foundations for support in the form of fellowships and Eli Lilly and Co. for support in the form of a granteeship.

Notes and References

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- 7) Obtained from Sachem, Austin, TX.
- 8) TLC was carried out on aluminum-backed silica plates, eluting with n-butanol/ethanol/water (5:4:3 v/v) and visualizing with MeOH/AcOH/H₂SO₄/p-anisaldehyde (200:20:10:1) followed by charring.
- 9) While working with a 60:40 mixture of mono- and disubstituted derivatives is not ideal, this method to obtain secondary side samples offers much higher yields than the previous method (Ueno, A. and Breslow, R. *Tetrahedron Lett.* 1982, 23, 3451), which yields almost exclusively mono-. To confirm that there is not a qualitative difference between these samples, we prepared 2 using the "old" method. Rate measurements made using this sample of 2 at pH's 7 and 9 gave rates only ≤ 15% slower than reported in Figure 1.
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SYNTHESIS AND TRANSACYLATING ACTIVITY OF ISOMERIC Co(III)-CYCLODEXTRIN ARTIFICIAL METALLOENZYMES

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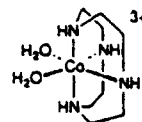
Although the cyclen-Co(III) complex has exhibited amongst the greatest rate accelerations in acyl and phosphoryl transfer reactions, this catalytic unit has not been used previously in the design of an artificial metalloenzyme. For this study, β -cyclodextrin derivatives of cyclen-Co(III) with attachments to the primary and secondary sides of the cyclodextrin torus were synthesized. The primary-side cyclodextrin-cyclen-Co(III) conjugate accelerates the hydrolysis of *p*-nitrophenylacetate by a factor of 1000 (pH 7.0, 25°C) in comparison with the water-catalyzed reaction. Maximum reactivity occurs at pH 7, consistent with the known pK_a values and hypothesized mechanism of action of Co(III) complexes. The secondary-side cyclodextrin-cyclen-Co(III) conjugate is less reactive towards *p*-nitrophenylacetate hydrolysis under saturating conditions. Reactivities towards an azide, a phosphonate and a phosphate triester were in each case less than five times greater than the buffer-catalyzed rate.

INTRODUCTION

Metal ions can catalyze a variety of reaction types, either by super-acid catalysis (including metal ion-bound hydroxide mechanisms) that has a directional or template effect, or by acting as a carrier of electrons in the catalysis of redox reactions. These properties of metal ions are exploited by enzymes and other proteins. Metalloenzymes are often involved in biochemical acyl, and always in phosphoryl, transfer reactions. Most of the enzymes which act on nucleic acids or nucleotides require a divalent metal ion (typically Zn^{2+} or Mg^{2+}) for optimum activity. Whereas enzymes demonstrate such desirable properties as large rate enhancements, substrate selectivity, activity under neutral aqueous conditions and turnover behavior, virtually all enzyme mimics studied to date succeed in mimicking only one or two of these properties at a time. The compounds described in this paper are no exception, but we are encouraged by their unique activity under neutral conditions.

One way of improving the specificity of metal-catalyzed processes is to attach the reactive metal ion center to a molecule that can selectively bind substrate molecules. Cyclodextrins (CDs) have been used for this purpose, as they have a hydrophobic cavity and can form inclusion complexes. Also, these cyclic oligomers of glucose can be modified on both primary and secondary sides, making them versatile molecules for this purpose. CD-based metalloenzyme mimics have been

reported to catalyze carbon dioxide hydration,¹ phosphotriester hydrolysis,² activated ester hydrolysis,³ furin oxidation⁴ and decarboxylation.⁵ The cyclen-Co(III) complex **1** has exhibited amongst the greatest rate accelerations in acyl and phosphoryl transfer reactions; accordingly, we have synthesized two cyclen-Co(III) complexes positioned alternately on the primary and secondary sides of β -CD.⁶



RESULTS AND DISCUSSION

Synthesis

6-Deoxy-6-monotosyl- β -CD is a useful intermediate for functionalizing β -CD on its primary side. The tosylation reaction has been carried out both in dry pyridine⁷ and in a biphasic mixture of diethyl ether and 0.1 M aqueous NaOH solution.⁸ In both procedures, the reaction yields a mixture of monotosylate (major product), ditosylates and unreacted β -CD. In our hands, the first method results in better yields. Repeated recrystallizations from warm water (60°C) did not remove the ditosylate impurity completely. Therefore, this tosylate mixture was reacted with cyclen (1,4,7,10-tetraazacyclododecane; **3**), prepared by following the literature procedures⁹ with some modifications. A

* Author for correspondence.

similar reaction of β -CD monotosylate with cyclam (1,4,7,11-tetraazacyclotetradecane) has been reported previously,¹⁰ but without experimental detail. Purification of the 6-cyclenyl derivative (4) was accomplished using CM-Sephadex ion-exchange chromatography, eluting with an NH_4HCO_3 linear gradient. The ditosylate impurity in the 'monotosylate' sample, when reacted with an excess of cyclen, will form a dicyclenyl-CD that can be separated easily from the monosubstituted product. The $\text{p}K_a$ values of cyclen are >1 , 1.73, 9.7 and 10.7.¹⁰ Therefore we predict the monocyclen compound to be +2 charged and the dicyclen compound to be +4 charged at pH 7. A solid sample of 6-deoxy-6-cyclenyl- β -CD was obtained after lyophilization, whose NMR and mass spectra and microanalytical characterization were supportive of the structure assignment.

When β -CD is treated with *m*-nitrophenyl tosylate at pH 10, the isolated organic product is almost exclusively the 3-deoxy-3-tosyl derivative.^{12a} On heating with NH_4HCO_3 in aqueous solution, the mannoepoxide 8 forms. This has been the most useful synthetic intermediate for the secondary side derivatization (alternative, higher yielding syntheses of the secondary side tosylate have been reported¹³). The literature procedure affords an epoxide that is $>50\%$ salt by weight, which has been used successfully for further reactions.^{12b} We have effected a 'desalting' of the CD-epoxide by passing an aqueous solution through an Amberlite MB-3 column (a mixture of cation- and anion-exchange resins). Lyophilization afforded a fluffy white product that was shown to be 'salt free' by elemental analysis. Epoxide 8 is not as reactive as the primary-side tosylate, probably for steric reasons; nucleophiles must approach from the inside of the CD cavity to open the epoxide ring. When 8 was reacted with excess cyclen at 100–110°C, substitution took place together with some hydrolytic opening of the epoxide ring. As with the primary-side derivative, compound 9 was purified by ion-exchange chromatography. Again, the NMR and mass spectra and microanalysis data are supportive of the structure assignment.

The preparations of various Co(III) complexes are documented in the literature.^{14,15} Our target Co(III) complexes were the diaqua forms. These complexes have been prepared mainly via two routes: by conversion of the dinitro complex to the dichloro complex followed by hydrolysis, or by preparation of the carbonate complex followed by direct hydrolysis. Carbonate complexes treated with concentrated acids bearing non-nucleophilic counterions can be converted in the diaqua complexes directly. However, reactions under strongly acidic conditions are sometimes not applicable to the preparation of CD derivatives because the glycosidic linkages are hydrolyzed with strong acids, especially when heated.¹⁶

We first reproduced the complex formation reaction

using cyclen itself. As a stable source of Co(III) with labile ligands, sodium triscarbonatocobaltate(III) was prepared by oxidizing Co(II) with H_2O_2 in the presence of NaHCO_3 .¹⁷ The olive-green complex is stable for a few weeks if properly kept dry. This complex, when reacted with cyclen hydrochloride (as described for the analogous cyclam complex¹⁸), gave the carbonate complex as pink microcrystals. The same reaction was then repeated using 4, affording carbonate complex 5 in 89% yield. The literature procedure for conversion of the cyclam-carbonate complex to the dichloro complex suggests heating for 1 h on a steam-bath.¹⁸ We have found that 5 min at 65°C is sufficient to complete the conversion of the carbonate complexes into the corresponding dichloro complexes. As with the cyclen-carbonate complex, a suspension of 5 in methanolic HCl, when heated at 60–65°C for 5 mins, forms the dichloro complex (6). Lyophilization affords 6 as a pink fluffy solid. The UV spectra of the cyclenyl and primary-CD-cyclenyl dichloro complexes were identical at $\lambda > 300$ nm (see Table 1). In addition, the fast atom bombardment (FAB) mass spectrum and the elemental analysis were consistent with the structure assignment (6). The dichloro complex is unstable in aqueous solutions, hydrolyzing to the mono-aqua-chloro complex. Dichloro complex 6 was therefore isolated as a hygroscopic purple powder.

The aqua complex of cyclen-Co(III) (i.e., 1) was obtained by hydrolysis of the dichloro complex; this was achieved simply by passing an aqueous solution of the dichloro complex through a strong anion exchange column followed by acidification and then trituration using diethyl ether. We found that Dowex resin (OH^- form) works very well for the cyclen compound, but that β -CD interacts strongly with the resin (inclusion of the aromatic portion of the resin is a possibility); in fact, no CD derivative could be eluted from the ion-exchange column. We therefore switched to a Sephadex resin (QAE-Sephadex) and were able to obtain the hydrolysis product as a solid material; however, the UV spectrum was different from that of the non-CD complex. In order to show that decomposition had not occurred during the ion-exchange process, another method of conversion to the aqua complex was examined. The carbonate-Co(III) complexes of the

Table 1. UV data ($\lambda_{\text{max}} \pm 1$ nm of the Co(III) complexes of cyclen derivatives in water

Ligand	Non-CD	Primary-CD	Secondary-CD
Carbonato	530	530	531
Dichloro	560	562	— ^a
(Di)aqua	504	524	508
Aqua-hydroxo	522	534	522

^a The secondary-side Co(III) dichloro complex hydrolyses rapidly to the diaqua complex.

tetraamine ligands hydrolyze almost immediately to the aqua complexes when treated with acids. The color change due to hydrolysis is apparent and can be also followed by UV spectrophotometry. As expected, on acidification the cyclen-carbonato complex changes color immediately. However, the same color change is not observed for the CD derivative. The UV spectra indicate a hydrolysis-induced spectral shift only to 524 nm. Since no other potential external ligand exists in the solution that is not also available to the parent

complex, we assign structure 6 to the compound obtained in this way. We postulate that one of the primary hydroxyl groups of an adjacent glucose unit is involved in coordination to the Co(III) cation. In fact, coordination to CD by metal ions has been shown previously for Mn^{3+} and Cu^{2+} ,¹⁹ and recently for Co(III) itself.²⁰

With some modifications, the yield of 6 was improved. It was found that the final ion exchange can be avoided altogether if the carbonato complex has

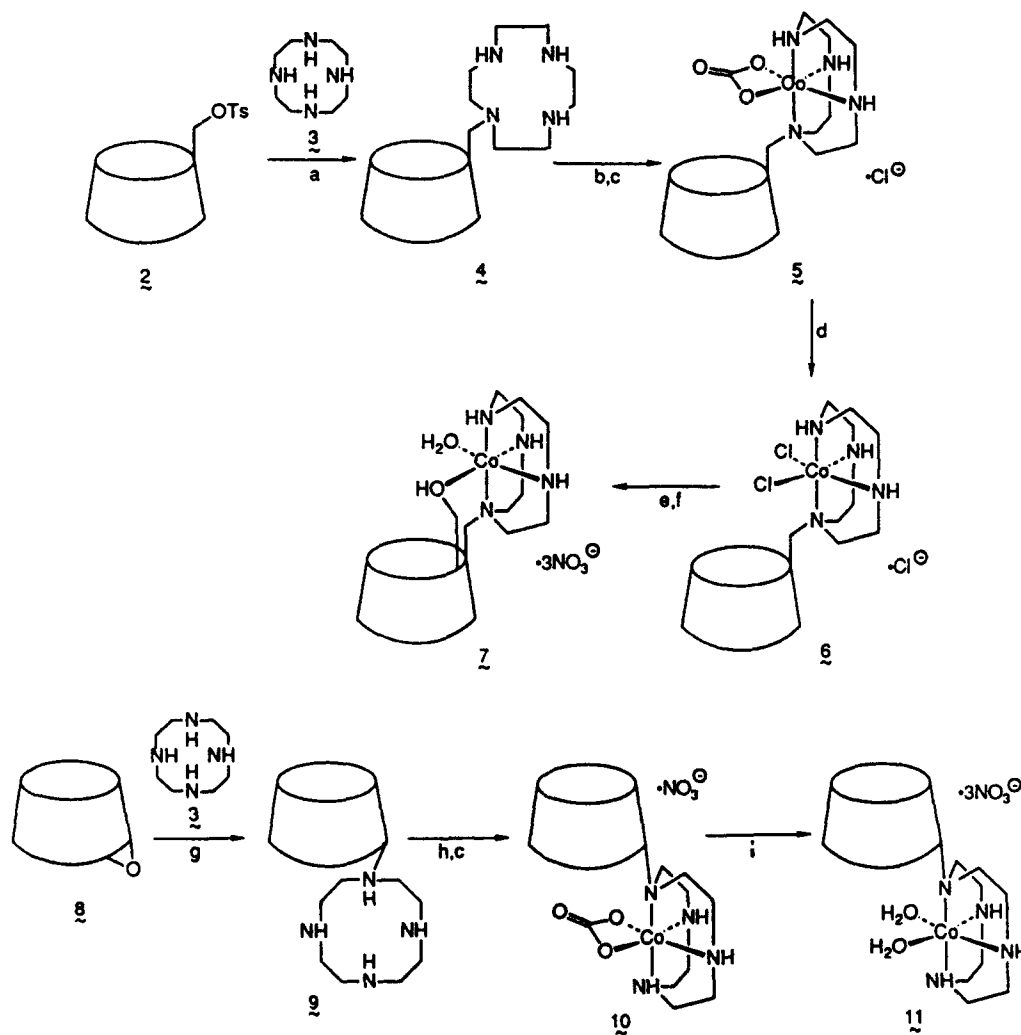


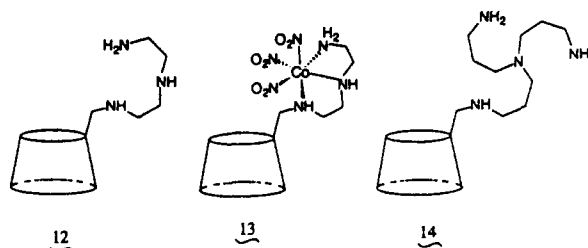
Figure 1. Synthesis of CD-cyclen-Co(III) complexes. (a) DMF, 80°C, CM-Sephadex (HCO_3^- form); (b) aq. HCl; (c) $Na_3[Co(CO_3)_3]$; (d) HCl-MeOH; (e) QAE-Sephadex (OH^- form); (f) acidification (HNO_3); (g) DMF, 105°C; (h) aq. HNO_3 ; (i) methanolic HNO_3 .

nitrate or perchlorate as the counterion simply by warming an acidic (acidified with HNO_3 or HClO_4) solution in MeOH for 5 min; conversion to the aqua complex is achieved efficiently. By this method we were able to prepare 0.8 g of the primary-side complex.

Preparation of the Co(III) complex with the corresponding secondary-side cyclen derivative was achieved similarly, although with an important modification (Figure 1). Carbonato complex 10 can be prepared from the corresponding triscarbonato complex exactly as described for the primary-side complex. MeOH-HCl treatment, however, produced some interesting results. The dichloro compound is purple, but reaction of the secondary-side derivative produced instead a bright red product. In methanol, the dichloro compound is stable, but when dissolved in water it immediately changes color. The λ_{max} of the resulting species was found to be 504 nm, the same as for the diaqua complex. In fact, it was observed that when an attempt was made to collect the solid purple dichlorides complex by filtration, the color changed from purple to red; the solid seemed to be hydroscopic and it hydrolyzed as it absorbed water from the air. Larger amounts of the secondary-side aqua complex were prepared by direct hydrolysis of the carbonato complex in MeOH- HNO_3 . More importantly, the reactivities of secondary-side aqua samples differ, depending on the method of isolation.

Our first communication of this work⁶ noted that the secondary-side complex obtained by hydrolysis of the dichloro complex, followed by hydrolysis on a QAE-Sephadex column, was unreactive towards activated ester and carbonate substrates. We now report that QAE-Sephadex chromatography itself, by an as yet undetermined reaction, results in the reactivity loss. Alternatively, direct hydrolysis of the carbonato nitrate complex with methanolic HNO_3 affords a sample that is spectroscopically identical with, but more reactive than, the QAE-Sephadex-treated material. Thus, the kinetic results reported in this paper are those obtained using the new synthesis method. This effect is not observed for the primary-side complex.

In an attempt to prepare a primary-CD-Co(III) complex with at least two aqua ligands coordinated to the Co(III) center (as required for catalytic activity in phosphate hydrolysis²¹), primary-dien-CD (12) was synthesized by the reaction of dien (diethylenetriamine) with primary-CD-tosylate. It was thought that since the Co(III) complex of dien bears three water ligands, even if one of the H_2O molecules was displaced by a CD hydroxyl group there would be two more labile ligands as required to form the cyclic phosphacohalt intermediate. We have been successful in preparing the dien-Co(III) complex, although only in the trinitro form (i.e. 13). We have found that the parent trinitro complex can be converted into the trischloro complex only under strongly acidic conditions. Therefore, although 13 was prepared successfully, conversion



attempts with 13 failed because significant acidic hydrolysis of the CD took place.

As part of this work, we also prepared the primary-CD derivative of tris(3-aminopropyl)amine (TRPN). Co(III) complexes of this ligand have been studied recently and found to have exceptional reactivity towards phosphodiesterases such as bis-*p*-nitrophenylphosphate.²² The polyamine ligand TRPN was synthesized using literature procedures.²³ Primary-TRPN-CD (14) was then synthesized and characterized successfully. Two different attempts were made to prepare a Co(III) complex, but each failed. In related work, we have likewise found that anthrylmethyl substitution on TRPN results in an inability to form Co(III) complexes. It appears that when the amine is substituted, our complexes cannot form. Hence, although cyclen as a ligand does not afford Co(III) complexes of maximum activity, it seems to be (at present) the optimum compromise between activity and the ability to synthesize the Co(III) complex in the first place.

Circular dichroism studies

Additional support for our structural assignment came from spectropolarimetric work done with complexes 7 and 11. The primary-side complex showed one positive peak at $19\,500\text{ cm}^{-1}$ in the first d-d absorption band region, whereas the secondary-side complex showed three peaks in the same region (Figure 2). As expected, circular dichroism contributions due to the cyclodextrin unit on the primary side, although mainly lined with achiral methylene carbons, are larger as the Co(III) center is fixed at a shorter distance from the CD by hydroxymethyl coordination. The observed secondary-side induced circular dichroism is less, suggesting the absence of a direct coordination of Co(III) center to the CD. It is also interesting that the circular dichroism spectrum for 11 is very similar to the published²⁰ spectrum of Co(III)en_2 complexed to the CD secondary side, CD supplying two vicinal hydroxyls of a glucose residue as a bidentate ligand.

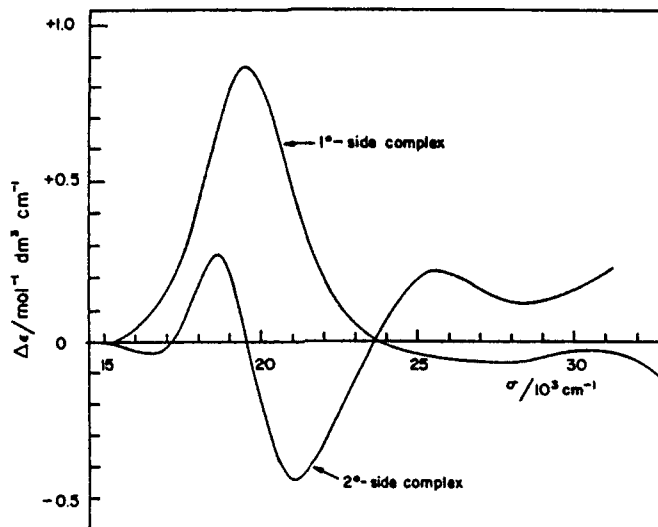


Figure 2. Circular dichroism spectra of primary and secondary side complexes

Reactions with ester, carbonate, amide and phosphate substrates

The reactivity of compounds 7 and 11 was evaluated with the activated substrates shown in Figure 3: *p*-nitrophenylacetate (PNPA), bis-*p*-nitrophenylcarbonate (BNPNC), *p*-nitrotrifluoroacetanilide (PNTFAA), bis-*p*-nitrophenylphenylphosphonate (BNPPPP) and bis-*p*-nitrophenylethylphosphate (BNPEP). Although activated substrates may not be good surrogates for the more interesting unactivated varieties, it is unlikely that an enzyme mimic inactive towards these reactants will prove active towards, e.g., an ethyl ester. Because the reactions of *p*-nitrophenyl substrates are easy to monitor experimentally, and because they do provide a yardstick for comparison against previously published work, we employed them in the current study.

As shown in Table 2, the transacylation reaction of PNPA bound to 7 at neutral pH is faster than that derived from the buffer. As both buffer and 7 are present in excess, pseudo-first-order rate constants are obtained. Because the reaction of PNPA is strongly accelerated by buffer whereas that of PNPA-7 is not, the intracomplex rate advantage increases with decreasing buffer concentration. Moreover, the reactivity of Co(III) complexes is well known to be decelerated by buffer owing to a reversible complexation that effectively decreases the concentration of the reactive aquahydroxo species. Thus, the intracomplex reaction is almost twice as fast with 0.1 M than with 0.2 M buffer. Whereas the zero buffer rate can be safely extrapolated for the uncomplexed reaction as

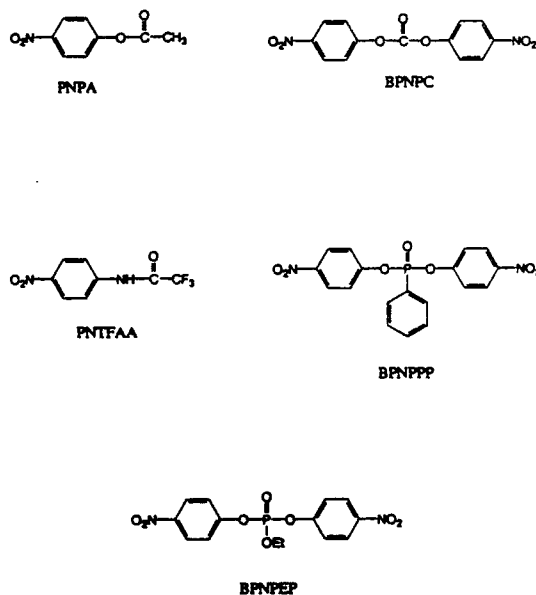


Figure 3. Structures of substrates used in this study

$1.3 \times 10^{-6} \text{ s}^{-1}$, extrapolation of the intracomplex reaction to zero buffer is more tenuous. The intracomplex rate advantage thus calculated ranges from 1000 to 1400-fold, with the former comparison the

Table 2. pH and buffer effects in PNPA reactions with 7^a

[7] (mM)	pH	[Buffer] (M)	k_{buffer}	k_7	k_7/k_{buffer}
5	7	0.2	0.5	7.3	15
	7	0.1	0.26	13	49
	7	0.0 ^b	0.013	(18)	1000–1400
	7	0.1	—	1.9 (0.1 M cyclohexanol)	
1	6	0.05	0.01	0.20	20
	7	0.05	0.028	2.0	75
	8	0.05	0.24	2.8	12
	9	0.05	0.95	5.5	5.8

^a All rate constants are $k_{\text{obs}} \times 10^4 \text{ s}^{-1}$. [PNPA] = 5 μM . The buffer materials used are listed under Experimental.

^b Obtained by extrapolation to zero buffer concentration.

more secure one. This proves to be the largest acceleration seen to date at pH 7 for a cyclodextrin–metal conjugate, and is attributable to the high reactivity of the cyclen–Co(III) complex. The acceleration is not due simply to reaction with 4, which demonstrates only an 8.6-fold acceleration over buffer under these conditions.²⁴

Table 2 also indicates a bell-shaped pH–rate profile for the intracomplex reaction (while comparisons of zero buffer rates would be much preferable, the amounts of 7 required for extrapolation were prohibitive). The apogee occurs at pH 7, consistent with two facts: (1) the *cis* water molecules on the cyclen–Co(III) complex have pK_a values of *ca* 6 and 8; and (2) it is the singly deprotonated (i.e. the aquohydroxo) form that shows the greatest reactivity. It is precisely this set of properties that portended the utility of Co(III) complexes in artificial metalloenzyme design.

Table 3 summarizes the effect of CD conjugate concentration on the reaction rate. Two conclusions may be drawn. First, the secondary-side conjugate achieves saturation (by 3 mM) before the primary-side conjugate does; this is consistent with an observed preference of *p*-nitrophenyl compounds to bind at the secondary-side

of cyclodextrin itself. Second, although the primary-side conjugate binds PNPA more weakly, it is nonetheless more reactive. Although we might deliberate on the origin of this observation, the rate ratio is really too small to warrant such speculation.

Bis-*p*-nitrophenylcarbonate (BPNPC) is one of the substrates most commonly used with carbonic anhydrase models. Both of the Co(III) complexes that we have prepared had some promotional activity towards BPNPC. Carbonate is a very good ligand for Co(III), and catalysis of CO₂ hydration by Co(III) complexes has been reported.²⁵ Again, with this substrate the primary-side complex was more reactive. Table 4 summarizes the reactions of 7 with BPNPC at various pH values. It is important to note that, although several buffering materials were used to obtain k_{buffer} rates at different pH, no corrections have been made (such as extrapolation to zero buffer concentration). Nevertheless, because the intracomplex reactions are much faster than the buffer reactions, a bell-shaped pH–rate profile

Table 3. Effect of changing Co(III) complex concentration in PNPA reactions at pH 8.0^a

[CD] (mM)	k_7	k_{11}
1.0	4.8	2.4
2.0	6.8	3.0
3.0	8.4	3.5
4.0	9.7	3.5

^a Buffer: 0.1 M bis-trispropane. All rate constants are $k_{\text{obs}} \times 10^4 \text{ s}^{-1}$. [PNPA] = 5 μM .

Table 4. Reactions of BPNPC with 7^{a,b}

pH	[Buffer] (M)	k_{buffer}	k_7	k_7/k_{buffer}
6	0.1	11	76	7.1
6.5	0.1	15	150	10
7	0.1	10	290	29
7.5	0.1	9.5	370	38
8	0.1	30	600	20
8.5	0.1	17	380	22
9	0.1	120	400	3.4

[7] = 2 mM.

^a All rate constants are $k_{\text{obs}} \times 10^4 \text{ s}^{-1}$. [BPNPC] = 5 μM .

^b The buffer materials used are listed under Experimental. Buffer rates shown are those for that buffer at 0.1 M concentration.

is observed. Again, this is consistent with the reaction proceeding via the aquohydroxo Co(III) species.

The reactions of 7 and 11 with amide (PNTFAA), phosphonate (BPNPPP) and phosphate (BPNPEP) substrates were also examined. Although rate increases over the buffer-catalyzed rates were observed in every case, the intracomplex advantage was never more than fivefold (which occurred using 4 mM 7 with BPNPPP at pH 8). *p*-Nitrophenylphosphate, which is of interest because of its structural analogy to nucleoside monophosphates, reacts about 20 times more slowly with 7 than with 1. It therefore appears that 7 and 11 show little or no intracomplex rate advantage towards these functional groups, even though non-CD Co(III) complexes demonstrate activity towards each substrate type. The most likely explanation is that the intracomplex reaction cannot achieve the conformation required for productive metal–functional group interaction. This is an issue that can be addressed; structure–reactivity relationships are, in principle, possible using synthetic catalysts; the structures of these compounds are known.

CONCLUSION

Although the cyclen–Co(III) complex has exhibited amongst the greatest rate accelerations in acyl and phosphoryl transfer reactions, this catalytic unit has not been used previously in the design of an artificial metalloenzyme. For this study, we have synthesized β -cyclodextrin derivatives of cyclen–Co(III) with attachments to the primary and secondary sides of the cyclodextrin torus. The primary-side cyclodextrin–cyclen–Co(III) conjugate accelerates the hydrolysis of *p*-nitrophenylacetate by a factor of 1000 (pH 7.0, 25 °C) as compared with the water-catalyzed reaction. Maximum reactivity occurs at pH 7, consistent with the known pK_a values and hypothesized mechanism of action of Co(III) complexes. The secondary-side cyclodextrin–cyclen–Co(III) conjugate is less reactive towards *p*-nitrophenylacetate hydrolysis under saturating conditions, perhaps because of strain that requires the metal to point away from the CD cavity (as predicted by space-filling models). Reactivities towards an amide, a phosphonate and a phosphate triester were smaller. Artificial enzymes that can act on an unactivated substrates remain elusive but important targets. It appears likely that a hydrolytically active Co(III) complex attached to a strong and selective binding-cavity bearing molecule may well be a good candidate for this purpose.

EXPERIMENTAL

General. Melting points were taken on an electrothermal melting point apparatus and are uncor-

rected. Microanalyses were carried out at Canadian Microanalytical Services (New Westminster, BC). Mass spectra were obtained by use of a Kratos-30 mass spectrometer. FT-NMR spectra were obtained at 11.75 T (500 MHz) or 7.0 T (300 MHz). UV spectra were obtained on a Hewlett-Packard Model 8451A diode-array spectrophotometer; all wavelength data reported are ± 1 nm. 6-Monotosyl- β -cyclodextrin (2) was prepared as described previously.⁷ Most of the chemicals used in this study were obtained from Aldrich Chemical (Milwaukee, WI). Biological buffers (pH buffer: 6/MES, 6.5/HEPES, 7–8/bis-trispropane, 8.5–9/CHES) and *p*-nitrophenylphosphate were obtained from Sigma Chemical (St. Louis, MO).

Reactions that produced *p*-nitrophenolate were followed by measuring the change in absorbance at 398 nm and 25 °C. For the reactions carried out at pH 6 and 6.5, the change in absorbance at 340 nm was followed (λ_{max} of *p*-nitrophenol). *p*-Nitroacetanilide reactions were followed at 400 nm. Reactions were monitored to >95% completion; pseudo-first-order behaviour was observed in most of the reactions, one important exception being that of *p*-nitrophenylphosphate hydrolysis.

6-Deoxy-6-(1',4',7',10'-tetrazacyclododecyl)- β -cyclodextrin (4). A solution of the 6-monotosyl derivative of β -cyclodextrin (1.3 g, 1.0 mmol) and cyclen (1,4,7,10-tetraazacyclododecane; 695 mg, 4.0 mmol) in dry DMF (4 ml; stirred with KOH and distilled from BaO) was heated in a sealed tube at 90 °C for 24 h. The reaction mixture was cooled, DMF was removed under reduced pressure and the residue was dissolved in water (2 ml) and added dropwise to ethanol (40 ml). The precipitated CD-containing compounds were collected by filtration, dissolved in 0.05 M NH_4HCO_3 buffer (25 ml) and applied to a CM-Sephadex cation-exchange column (30 \times 7 cm i.d.). A linear gradient of NH_4HCO_3 (from 0.05 to 0.5 M) was used for elution. The fractions (25 ml each) were checked by TLC; CD-containing spots were made visible using a MeOH–AcOH–H₂SO₄–*p*-anisaldehyde (200:20:10:1) spray and developing with heat. Fractions 50–110 were combined and lyophilized to afford a fluffy white solid (4; 675 mg, 52%). ¹H NMR (D₂O): δ 2.35–3.10 (m, 12 H, azamacrocyclic), 3.24–4.12 (m, 42 H, H₂, H₃, H₄, H₅, H₆ and 4 H azamacrocyclic), 4.87–5.15 (m, 7 H, H₁). FAB mass spectrum: m/z 1290 ($M^+ + 1$). Analysis: calculated for C₅₀H₈₈N₄O₃₄·6H₂O·NH₄HCO₃, C 41.49, H 7.17, N 4.74; found, C 41.71, H 7.12, N 4.7%.

6-Deoxy-6-(1',4',7',10'-tetraazacyclododecyl)- β -cyclodextrin(*N*^{1'},*N*^{4'},*N*^{7'},*N*^{10'})carbonatocobalt(III) chloride (5). To a solution of 4 (500 mg, 0.39 mmol) in 1.2 M HCl (1.0 ml) was added sodium triscarbonato-Co(III) (159 mg, 0.44 mmol) in portions. After CO₂

evolution had ceased, the solution was warmed to 66 °C for 5 min. The solution was cooled to room temperature, filtered and acetone (50 ml) was added. The product separated as pink microcrystals (5; 498 mg, 89%). UV (λ_{max} , H₂O): 368, 530 nm. ¹H NMR (D₂O): δ 2.45–4.42 (m, 16 H, azamacrocyclic); 4.2 H, H₂, H₃, H₄, H₅, H₆), 4.80–5.18 (m, 7 H, H₁). FAB mass spectrum: m/z 1347 ($M^+ - \text{CO}_3 - \text{Cl}$).

6-Deoxy-6-(1',4',7',10'-tetraazacyclododecyl)- β -cyclodextrin($N^{1'}$, $N^{4'}$, $N^{7'}$, $N^{10'}$)bischlorocobalt(III) chloride (6). Compound 5 (498 mg, 0.34 mmol) was suspended in methanol (8 ml) and conc. HCl (1.0 ml) was added. The mixture was warmed to 65 °C for 5 min. A purple solid precipitated on cooling (6; 454 mg, 90%). UV (λ_{max} , H₂O): 390, 562 (unstable in aqueous solutions). FAB mass spectrum, m/z 1347 ($M^+ - 3\text{Cl}$). Analysis: calculated for $\text{C}_{50}\text{H}_{88}\text{Cl}_3\text{CoN}_4\text{O}_{34} \cdot \text{NH}_4\text{Cl} \cdot 7\text{H}_2\text{O}$, C 36.75, H 6.54, N 4.28, Cl 8.68; found, C 37.05, H 6.51, N 3.89, Cl 8.71%.

6-Deoxy-6-(1',4',7',10'-tetraazacyclododecyl)- β -cyclodextrin($N^{1'}$, $N^{4'}$, $N^{7'}$, $N^{10'}$, O^{6B})aquacobalt(III) nitrate (7). Compound 6 (400 mg, 0.28 mmol) was dissolved in water (5 ml) and the solution was applied to a QAE-Sephadex anion-exchange column (OH[−] form, 15 × 3 cm i.d.) and eluted with water. The pink eluate was concentrated under reduced pressure to 5 ml. The solution was acidified with 4 M HNO₃ (1 ml) and triturated with Et₂O until the product separated as a reddish powder, which was filtered and then dried *in vacuo* at room temperature (7; 251 mg, 59%). ¹H NMR (D₂O): δ 2.30–4.15 (m, 16 H, azamacrocyclic); 4.2 H, H₂, H₃, H₄, H₅, H₆), 4.90–5.25 (m, 6 H, H₁), 5.28–5.45 (br d, 1 H, H₁). UV (λ_{max}): 366, 522 nm (0.1 M HNO₃), 368, 540 nm (pH 7 buffer), 362, 576 nm (0.1 M NaOH). FAB mass spectrum: m/z 1347 ($M^+ - \text{H}_2\text{O} - 3\text{NO}_3$). Analysis: calculated for $\text{C}_{50}\text{H}_{90}\text{CoN}_4\text{O}_{44} \cdot 6\text{H}_2\text{O} \cdot \text{HNO}_3$, C 34.53, H 5.97, N 6.44; found, C 34.80, H 6.12, N 6.29%.

Direct conversion of the primary-carbonatocobalt(III) complex into the aqua complexes. We have found out that carbonato-cyclenyl-CD-Co(III) complexes can be converted directly into the aqua complexes with high yields. Both primary- and the secondary-side complexes can be reacted in this way. This procedure will be exemplified here with the primary-side complex. First, the carbonato complex was prepared in the nitrate or perchlorate form. The carbonato complex (0.8 g) was then suspended in MeOH (10 ml) and concentrated HNO₃ (0.5 ml) was added. The bright pink color of the aqua complex was observed immediately. To remove any dissolved CO₂, the solution was heated on a steam-bath for 2 min. The mixture was then cooled to room temperature and the

bright pink precipitate was collected by filtration. The precipitate was dissolved in water (30 ml) and lyophilized to afford a fluffy pink solid (0.585 g, 50%), identical in all respects with material prepared by initial conversion to the primary-side dichloro complex.

β -Cyclodextrin-manno-2,3-epoxide (8). The literature procedure^{12a} was used to prepare the epoxide. However, the white solid obtained in this way is a mixture of product and various salts. The salts were removed by passing an aqueous solution of the epoxide sample through a cation-anion-exchange column (Amberlite MB-3). The salt-free epoxide was obtained after lyophilization. TLC indicated the presence of small amounts of CD and of the diepoxide. Analysis: calculated for $\text{C}_{42}\text{H}_{68}\text{O}_{34} \cdot 5\text{H}_2\text{O}$, C 41.79, H 6.51, found, C 41.66, H 6.43%.

3-Deoxy-3-(1',4',7',10'-tetraazacyclododecyl)- β -cyclodextrin (9). To a solution of 8 (655 mg, 0.59 mmol) dissolved in dry DMF (4.0 ml) was added cyclen (1,4,7,10-tetraazacyclododecane; 400 mg, 2.3 mmol). The solution was heated at 100 °C for 48 h, then DMF was removed under reduced pressure. The residue was dissolved in water (2.0 ml) and added to EtOH (45 ml) dropwise. The resulting precipitate was collected by filtration, dissolved in water (50 ml) and then applied to a CM-Sephadex cation-exchange column (30 × 7 cm i.d.). Elution was performed using a linear gradient of NH₄HCO₃ buffer (0.05 to 0.6 M). Fractions were analyzed by TLC as described for compound 2. Appropriate fractions (55–100) were pooled, concentrated and lyophilized to afford the secondary-side derivative (9; 187 mg, 25%). ¹H NMR (D₂O): δ 2.50–3.15 (m, 12 H, azamacrocyclic), 3.19–4.08 (m, 4.2 H, H₂, H₃, H₄, H₅, H₆; 4 H azamacrocyclic), 4.85–5.22 (m, 7 H, H₁). FAB mass spectrum: m/z 1289 (M^+). Analysis: calculated for $\text{C}_{50}\text{H}_{88}\text{N}_4\text{O}_{34} \cdot \text{NH}_4\text{HCO}_3 \cdot 6\text{H}_2\text{O}$, C 41.49, H 7.17, N 4.74; found, C 41.11, H 7.12, N 5.06%.

3-Deoxy-3-(1',4',7',10'-tetraazacyclododecyl)- β -cyclodextrin($N^{1'}$, $N^{4'}$, $N^{7'}$, $N^{10'}$)diaquacobalt(III) nitrate (11). To a solution of 9 (415 mg, 0.32 mmol) in 1.2 M HNO₃ (0.80 ml) was added Na₃Co(CO₃)₃ · 3H₂O (132 mg, 0.37 mmol). After the effervescence had stopped, the mixture was heated at 60 °C for 5 min, cooled and filtered. The solid was washed with cold water (5 ml), then to the clear red, combined filtrate were added acetone (5 ml) and EtOH (50 ml). The precipitate was collected by filtration, suspended in MeOH (6 ml), and conc. HNO₃ (six drops) was added. The mixture thus obtained was heated at 60 °C for 4 mins and cooled to room temperature. The reaction vessel was evacuated to remove dissolved CO₂, and the resulting precipitate was collected by filtration, dissolved in water (100 ml) and lyophilized to provide 11 as a red

fluffy solid (213 mg, 24%). UV (λ_{max}): 508 (0.1 M HNO_3), 522 nm (pH 7 buffer), 530 nm (0.1 M NaOH): ^1H NMR (D_2O): δ 2.20–4.40 (m, 16 H, azamacrocyclic, 42 H, H_2 , H_3 , H_4 , H_5 , H_6), 4.50–5.32 (m, H_1 , 7 H). FAB mass spectrum: m/z 1409 ($\text{M}^+ - 2\text{NO}_3 - 2\text{H}_2\text{O}$), 1347 ($\text{M}^+ - 2\text{H}_2\text{O} - 3\text{NO}_3$). Analysis: calculated for $\text{C}_{50}\text{H}_{92}\text{CoN}_7\text{O}_{45} \cdot \text{HNO}_3 \cdot 6\text{H}_2\text{O}$, C 34.49, H 6.08, N 6.43; found, C 34.28, H 5.93, N 6.80%.

6-Deoxy-6-(1',4',7'-triazheptyl)- β -cyclodextrin (12). To a solution of **2** (655 mg, 0.59 mmol) dissolved in dry DMF (4.0 ml) was added dien (1,4,7-triazheptane; 400 mg, 2.3 mmol). The solution was heated at 70 °C for 24 h, then DMF and most of the diethylenetriamine were removed under reduced pressure. The residue was dissolved in water (2.0 ml) and the solution was added to EtOH (45 ml) dropwise. The precipitate was collected by filtration, dissolved in water (50 ml) and applied to a CM-Sephadex cation-exchange column (30 \times 7 cm i.d.). Elution was performed using a linear gradient of NH_4HCO_3 buffer (0.05 to 0.5 M). Fractions were analyzed by TLC as for compound **4**. Appropriate fractions (50–90) were pooled, concentrated and lyophilized to afford the primary-side derivative (**12**; 187 mg, 25%). ^1H NMR (D_2O): δ 2.55–3.13 (m, 6 H, polyamine), 3.19–4.08 (m, 42 H, H_2 , H_3 , H_4 , H_5 , H_6 ; 2 H polyamide), 4.85–5.22 (m, 7 H, H_1).

6-Deoxy-6-[5'-N-(3'-aminopropyl)-1',5',9'-triazanonyl]- β -cyclodextrin (14). To a solution of **2** (1.3 g, 0.587 mmol) dissolved in dry DMF (4.0 ml) was added TRPN [5'-N-(3'-aminopropyl)-1,5,9-triazanone; 1.0 g, 2.3 mmol]. The solution was heated at 70 °C for 12 h, then DMF and some of the polyamide were removed by vacuum distillation. The residue was dissolved in water (6.0 ml) and added to EtOH (75 ml) dropwise. The precipitate was collected by filtration, dissolved in water (40 ml) and applied to a CM-Sephadex cation exchange column (30 \times 7 cm i.d.). Elution was performed using a linear gradient of NH_4HCO_3 buffer (0.05 to 0.8 M). Fractions were analysed by TLC as for compound **4**. Appropriate fractions (61–120) were pooled, concentrated and lyophilized to afford the primary-side derivative (**14**; 480 mg, 25%). ^1H NMR (D_2O): δ 2.52–3.11 (m, 16 H, polyamide), 3.20–4.11 (m, 42 H, H_2 , H_3 , H_4 , H_5 , H_6 ; 2 H polyamide), 4.83–5.19 (m, 7 H, H_1).

ACKNOWLEDGEMENTS

We are indebted to Professor Kazuaki Yamanari of the Faculty of Science, Osaka University, for measuring the circular dichroism spectra of our primary- and secondary-side Co(III) complexes. We thank the Office of Naval Research and the Petroleum Research Fund

for financial support of this work. Fourier transform (FT) NMR spectra were obtained using equipment funded in part by NIH Grant No 1. S10 R01458-01A1. A.W.C. thanks the A. P. Sloan Foundation for support in the form of a Fellowship and Eli Lilly and Company for support in the form of a Granteeship.

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Preassociating α -Nucleophiles¹

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Received October 11, 1991

Research on cyclodextrin (CD) transacylase mimics has been among the most fruitful in the artificial enzyme field. While most proteases function efficiently at pH 7.4, β CD itself is well-known to be inert at this pH; rather, it reacts rapidly with esters only when its secondary hydroxyl groups (pK_a 12.1) have begun to deprotonate.⁴ Thus, the synthesis of synthetic transacylases with reactivity at neutral pH presents itself as an important goal of practical significance. Toward this end, CDs have been prepared bearing imidazole as a group with reactivity at pH 7;⁵ pendant coordination complexes have likewise been employed.⁶ However,

(1) This paper is dedicated to Professor Ronald C. D. Breslow on the occasion of his 60th birthday.

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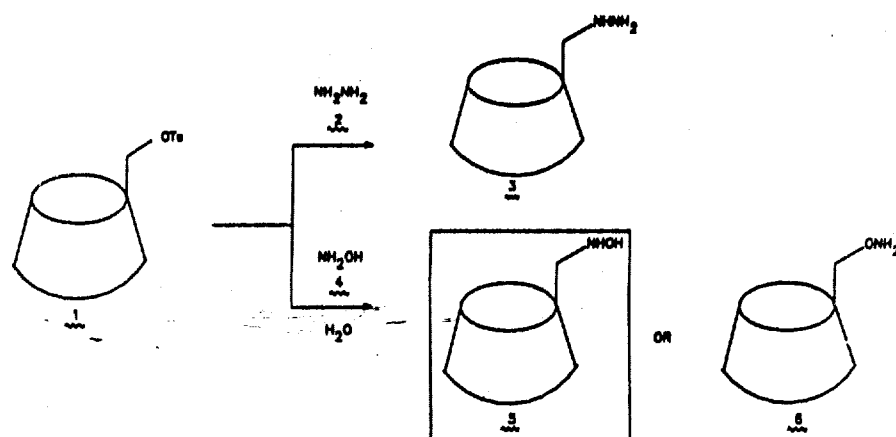
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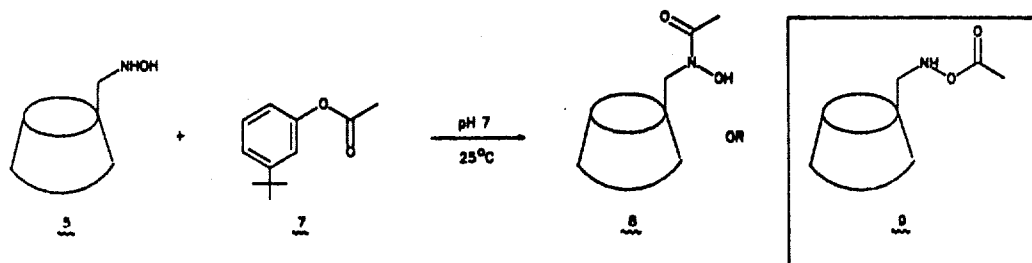
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Scheme I



Scheme II



as potential pendant groups, α -nucleophiles such as hydrazine or hydroxylamine offer unique properties. (1) In solution, α -nucleophiles show enhanced reactivity toward acyl transfer as compared to isosteric alcohols or amines.⁷ (2) Despite their greater reactivity toward acyl compounds, hydroxylamine (pK_a 5.97) and hydrazine (pK_a 8.0) are less basic than isosteric amines (pK_a 9–10) and thus exist in a reactive form near neutral pH. (3) Both hydroxylamine and hydrazine transacylate alkyl esters and amides. (4) Because they are physically small, pendant α -nucleophiles would necessarily reside proximal to the CD binding cavity. We now report on the syntheses, characterizations, and reactivities of β CDNHNH₂ and β CDNHOH.

Reaction of β CD-1^o-tosylate (1) in anhydrous hydrazine (2) at room temperature for 4 h, followed by precipitation from EtOH, gave the crude product (3) (Scheme I). Physically entrained NH_2NH_2 ⁸ was removed by reprecipitation from EtOH (5 \times), which gave 3 in 60% yield. In an analogous manner, reaction of 1 with a 6% aqueous solution of hydroxylamine (4) at 90 °C for 3 h, followed by multiple reprecipitation from EtOH, gave 5 in 36% yield. While either the N- or the O-alkylation product might have been formed, catalytic hydrogenation, which yielded β CDNH₂, and not β CD itself, confirmed the former. Notably for an unsymmetrically substituted CD derivative, 5 yields colorless plates (dec 207–210 °C) from water.⁹

Both β CDNHNH₂ and β CDNHOH are acylated rapidly by *p*-nitrophenyl acetate (pNPA) with saturation behavior. The reaction of pNPA (0.05 mM) fully complexed to 5 (10 mM) at pH 7.0 and 25 °C is faster than that with an equal concentration of CH_3NHOH ($k_2 = 1.0 \text{ M}^{-1} \text{ s}^{-1}$), demonstrating an effective RNHOH concentration of 37 mM. β CDNHOH is acylated as

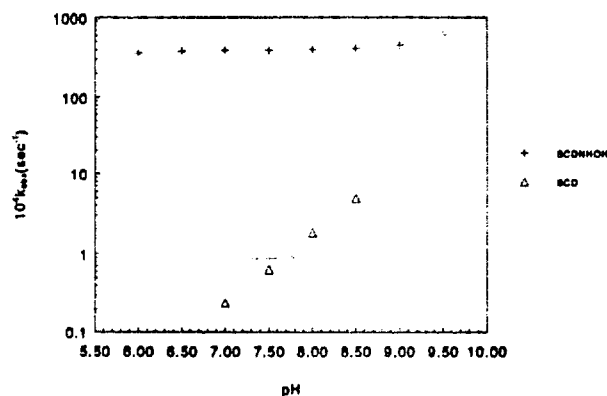


Figure 1. Reaction of pNPA with β CDs: effect of pH on k_{obs} (various buffers, 0.1 M).

efficiently at pH 7.0 as at pH 9.5; furthermore, the rate of acyl transfer is 1500 times faster than that afforded using equimolar β CD, which is not reactive under neutral conditions (Figure 1).

As shown in Scheme II, β CDNHOH binds and is acylated by a less activated ester (7) at pH 7.0 and 25 °C. The intracomplex reaction ($[5] = 20 \text{ mM}$; $[7] = 0.82 \text{ mM}$) occurs with a half-life of 7.5 min; a reference reaction (minimal DMSO added for solubility) with CH_3NHOH and β CD shows $t_{1/2} = 4.8 \text{ h}$, while the hydrolysis of 7 without added β CDNHOH occurs to less than 5% after 7 days. Once again, either N- or O-acylation, leading to 8 or 9, is possible. The ¹H NMR spectrum of the acylation product in DMSO-*d*₆ reveals a one-proton, D₂O-exchangeable triplet at δ 7.65. Decoupling experiments demonstrate one-bond coupling to a single, diastereotopic H-6 proton (δ 3.07) on the modified CD residue, which permits assignment as an NH proton, and thus an unambiguous assignment of the acyl enzyme mimic as 9. Because O-acylhydroxylamines hydrolyze more rapidly than structurally related esters, the deacylation kinetics of 9 are currently under investigation.

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(8) The 1:1 hydrazone between acetone and hydrazine yields methyl singlets at 1.72 and 1.81 ppm (D₂O); the 2:1 bis(hydrazone) yields methyl singlets at 1.68 and 1.91 ppm. The 1:1 hydrazone between acetone and β CDNHNH₂ yields somewhat broadened methyl singlets at 1.74 and 1.83 ppm. Likewise, the 1:1 oxime between acetone and hydroxylamine yields methyl singlets at 1.75 and 1.79 ppm; β CDNHOH does not form an oxime with acetone.

(9) Full synthetic details, with characterization data, are included in the supplementary material.

Acknowledgment. This work was supported by a grant from the Office of Naval Research. FT-NMR spectra were obtained with equipment funded in part by NIH Grant 1 S10 RR01458-01A1. L.E.F. acknowledges a faculty summer fellowship from the donors of the Petroleum Research Fund, administered by the American Chemical Society. A.W.C. thanks the A. P. Sloan and Dreyfus Foundations for support in the form of fellowships and Eli Lilly and Co. for support in the form of a granteeship.

Supplementary Material Available: Experimental details for the syntheses of 3 and 5 (4 pages). Ordering information is given on any current masthead page.